



## Beneficial effects of whey protein preloads on some cardiovascular diseases risk factors of overweight and obese men are stronger than soy protein preloads – A randomized clinical trial

Atefeh Tahavorgar <sup>a</sup>, Mohammadreza Vafa <sup>b, c, \*</sup>, Farzad Shidfar <sup>b</sup>, Mahmoodreza Gohari <sup>d</sup>, Iraj Heydari <sup>c</sup>

<sup>a</sup> School of Nutritional Sciences and Dietetics, Tehran University of Medical Sciences, Enqelab Square, Tehran, Iran

<sup>b</sup> Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Hemaat Highway, 1449614535, Tehran, Iran

<sup>c</sup> Endocrine Research Center (Firouzgar), Institute of Endocrinology and Metabolism, Iran University of Medical Sciences, Hemaat Highway, Tehran, Iran

<sup>d</sup> Department of Statistics, Iran University of Medical Science, Tehran, Iran

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### ABSTRACT

**Background:** The hypothesis that long term supplemental preloads of whey protein concentrate (WPC) and soy protein isolate (SPI) 30 min before the largest meal, will improve blood pressure (BP), fasting blood sugar (FBS) and lipid profile of overweight and obese men in their in free living condition was tested.

**Methods:** Forty - five men of 52, BMI = 25–40 kg/m<sup>2</sup>, after random allocation in WPC (n = 26) or SPI (n = 19) groups, drank 65 gr WPC or 60 gr SPI dissolved in 500 ml water 30 min before their ad libitum lunch for 12 weeks. Lipid profile and FBS were assessed before and after the study. Systolic and diastolic BP were measured before and after the study and every two weeks.

**Results:** After 12 weeks, mean changes between the groups were significant for SBP (p < 0.02), DBP (p = 0.001), apo A-I, apo B (p < 0.001), LDL (p = 0.015), HDL (p = 0.017). Within group mean changes of WPC were significant for reduction of DBP, FBS, apo B, VLDL, LDL, TG (p < 0.001), SBP, TC (p = 0.001), and for increase of apo A-I (p < 0.001) and HDL (p = 0.001) relative to baseline. In SPI group, mean changes were significant relative to baseline for decrease of SBP (p < 0.02), DBP (p = 0.001), apo B (p < 0.001), LDL (p = 0.015) and for increase of apo A-I (p < 0.001) and HDL (p = 0.017).

**Conclusion:** According to this study, WPC preloads at 30 min before ad libitum main meal, exert stronger beneficial effects than SPI preloads on BP, FBS and lipid profile of free living overweight and obese men after 12 weeks.

Trial registration: Iranian Registry of Clinical Trials: IRCT201109062365N3.

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### 1. Introduction

A leading cause of cardiovascular diseases is the accelerating increase in the prevalence of obesity with its related complications of hypertension, dyslipidemia, diabetes and atherosclerotic vascular disease [1]. Two major goals of dietary recommendations are to lower blood pressure (BP) and improve serum lipids, 2 of the

primary determinants of CVD risk [2]. In this regard, life style modifications such as losing weight, increasing physical activity, reducing the consumption of salt and saturated and trans fatty acids and increasing the consumption of fruits and vegetables have been consistently shown to improve BP, blood lipids and blood glucose [3,4]. On the other hand, limited studies have shown beneficial effects of high protein diets on BP, blood lipids [5–7] and glucose [8]. Studies show that the quality and quantity of dietary protein affect the plasma cholesterol levels [9]. Interestingly, the inverse association between dairy consumption and metabolic disorders [10] may be contributed to dairy-derived special components like calcium and other minerals, whey or casein proteins [11]. Current evidence demonstrates that ACE inhibiting peptides of

\* Corresponding author. Department of Nutrition, School of Public Health, Iran University of Medical Sciences, Hemaat Highway, 1449614535, Tehran, Iran.

E-mail addresses: [athvgr\\_ndt@yahoo.com](mailto:athvgr_ndt@yahoo.com) (A. Tahavorgar), [rezavafa@yahoo.com](mailto:rezavafa@yahoo.com) (M. Vafa), [f-shidfar@tums.ac.ir](mailto:f-shidfar@tums.ac.ir) (F. Shidfar), [mgothari@tums.ac.ir](mailto:mgothari@tums.ac.ir) (M. Gohari), [Irajheydari@gmail.com](mailto:Irajheydari@gmail.com) (I. Heydari).

**List of abbreviation**

ACE	angiotensin converting enzyme
BCAA	branched-chain amino acids
WPC	whey protein concentrate
SPI	soy protein isolate
FBS	fasting blood sugar
TC	total cholesterol,
TG	triglyceride
LDL	low density lipoprotein
HDL	low density lipoprotein
VLDL	very low density lipoprotein
Apo A-I	apolipoprotein A-I
Apo B	apolipoprotein B
BMI	body mass index
IPAQ	international physical activity questionnaire
SBP	systolic blood pressure
DBP	diastolic blood pressure
NASH	nonalcoholic steatohepatitis

whey protein, the by product of cheese producing, can be effective in decreasing hypertension [12]. On the other hand, beneficial effects of whey protein on lipid profile are contributed to inhibiting the expression of genes involved in fatty acid and cholesterol absorption and synthesis [13]. Furthermore, high concentrations of BCAAs (leucine, isoleucine and valine) in whey protein have beneficial effects on blood glucose [14]. Moreover, considering the fact that soy protein is rich in genistein, daidzein and glycitein isoflavones [15], it can reduce hypertension [16] and blood glucose [17]. Additionally, two main protein components,  $\beta$ -conglycinin and glycinin also have cholesterol lowering effects [18]. Despite numerous studies comparing effects of high protein diets from mixed sources, studies on the effects of long term supplemental WPC and SPI preloads before ad libitum food intake on metabolic risk factors in overweight and obese individuals without changing physical activity and dietary intake are scarce and similar studies have revealed controversial results [19–23]. According to similar interventions, whey protein either in concentrated or isolated form is mostly compared with carbohydrate as a control group [24,25] which masks the effects of high protein diets on metabolic factors as a confounding factor, thus it is not obvious whether the results are related to the high protein diet or to the specific characteristics of the protein type for instance whey or soy protein. Indeed, studies evaluating effects of soy protein intake and whey protein on apo A-I need more investigations and also understanding which protein sources are associated with lower CVD risk factors is important because substituting one protein for another may help individuals benefit from high protein diets.

Thus, the objective of the present study was to test the hypothesis that preloading free living overweight and obese men with supplemental WPC and SPI at 30 min before their ad libitum lunch meals (the largest meal of obese men) can decrease BP, FBS, TC, TG, LDL, HDL, VLDL, apo A-I and B.

## 2. Methods and materials

### 2.1. Study population and design

Volunteer employees of power plant generation of Karaj city were recruited to participate in the study by monthly bulletin. The study population was calculated 46 according to apo B which was increased to 52 to prevent 12% possible participant dropouts. A

total of 7 visits were included: one prior to the study for screening and baseline data collection and also 1 visit at the end of weeks 2, 4, 6, 8, 10 and 12 for completing questionnaires of 24-h dietary recalls, anthropometry and body composition measurements. Senior researcher completed all questionnaires to minimize mis-reporting. Of 85 volunteers, 52 men, age 30–65 yr, BMI = 25–40 kg/m<sup>2</sup> were recruited according to the following inclusion criteria: no cigarette smoking and alcohol consumption, no taking medication and supplements, no high amounts of caffeine consumption (>250–300 mg/d), no history of diseases or clinical problems increasing oxidative stress (injuries or burns), no allergy to soy/cow's milk and no severe weight changes within 3 last months (according to medical history of employees' records and their physical examination by the physician of the power plant). Exclusion criteria: changes in physical activities (PA), diets and also a compliance of 70% or lower for consumption of treatment beverages. Percent of consumed to distributed sachets were used to assess the compliance. At visit 1, eligible participants were randomly assigned to groups WPC or SPI (26 in each) using a convenience allocation. Individuals were instructed to deliver empty sachets in exchange for full ones at visits 2 to 12 for calculating compliance. All participants had ad libitum access to calorie and were asked to maintain their usual dietary intake and physical activity levels during the study. They were instructed to dissolve one sachet in 500 ml water and drink it 30 min before lunch meal (as the largest Iranian meal) every day. Aside from assessing compliance at biweekly visits, participants were observed directly at random visits at lunchtime in their workplaces to ensure the supplements were taken appropriately. They gave their written informed consent before participating in the study. The present study was conducted according to consolidated standards of reporting trials (CONSORT) guidelines [26] and has been registered in the Iranian Registry Clinical Trials (IRCT201109062365N3).

The Ethics Committee of Tehran University of Medical Sciences approved this study (Ethics Committee: 2011-06-17-13475-43649).

### 2.2. Treatment beverages

Preload proteins were WPC 80% (DMV, Netherlands) and SPI 90% (Red Crown, China) with almost similar color and texture. Sachets contained 67.5 g WPC and 60 g SPI (54 g effective compound as a protein/sachet). Calorie content of WPC and SPI sachets were 261.8 Kcal and 216 Kcal respectively. They were all closely matched for taste by strawberry flavor and sucralose (Vita Sweet, China) (0.2 gr and 0.1 gr in each sachet respectively), as a no-energy sweetener because sucralose is not metabolized in the body and has no effect on blood glucose or insulin secretion [27]. After packing in 4368 similar sachets for 84 days, they were numbered 1–84. The numbers were randomly divided into groups A and B (SPI and WPC respectively) and kept by research executive director until study complement. Hence, all groups were blinded for both participants and senior researcher. Protein concentration of treatment beverages was 13% for WPC and 12% for SPI.

### 2.3. Dietary intake and physical activity assessment

All 24-h dietary recalls were completed (1 weekday and 1 weekend day) before the study and at every 2 weeks (2, 4, 6, 8 and 12) [28]. Calorie and nutrient compositions were calculated using Nutritionist 4 software. PA was recorded by international physical activity questionnaire (IPAQ) and recorded as metabolic equivalent/wk (MET-Minute/Wk) [29] before the study, at the end of months 1, 2 and 3. MET values of 3.3, 4 and 8 were respectively considered for walking, moderate and vigorous intensity activities.

#### 2.4. Measurement of blood pressure assessment

Blood pressure was measured twice every 2–3 min using a standard mercury sphygmomanometer and the average was recorded. This measurement was repeated every two weeks, before and at the end of the intervention.

#### 2.5. Measurement of fasting blood sugar and lipid profile

Fasting blood glucose and lipids were measured before and after the intervention. A 10 ml fasting blood sample was taken via venepuncture from participants after a 12–14 h fasting state, at baseline and 12 weeks, to measure circulating FBS, TC, TG, HDL, LDL, apo AI, apo B. Samples were centrifuged within 30 min of collection for 10 min at 3500 RPM. Tests of TG, TC, LDL, HDL were analyzed by Hitach 717 and apo AI and B by Cobas Integra 400. Methods used for measuring variables were as follows: plasma glucose levels: enzymatic colorimeter method based on Glucose oxidase (Eli tech diagnostics kit, Indonesia,  $0.85 \times 10^{-3}$  mg/dL), apo A and B: enzymatic immunoturbidometry method (Tina – quant Apo lipoprotein kit, Germany), TC: enzymatic colorimeter (Eli tech diagnostics kit, Indonesia,  $1.53 \times 10^{-3}$  mg/dL), LDL: enzymatic Selective Protection (LDL-C L-Type kit, Germany, 1 mg/dL), HDL: Stable Liquid Reagen Immunoinhibition (HDL – C L-Type, Germany, 0.1 mg/dL), TG: enzymatic colorimeter (Eli tech diagnostics kit, Indonesia,  $1 \times 10^{-3}$  mg/dL) and VLDL: Fredlwald formula [30].

#### 2.6. Statistical analyses

All participants who completed the study were included in the data analysis. The data are normally distributed and statistical analysis was conducted using SPSS 17 for Windows (SPSS, Inc., Chicago, IL, USA). Data are assessed for normality by the Kolmogorov–Smirnov test. A paired *t* test was conducted to assess the effects of 12 week intervention with WPC or SPI preloads on BP, FBS, TC, TG, LDL, HDL, VLDL, apo AI and apo B. The within mean changes of parameters were assessed by calculating differences between the pre- and post intervention measurements. An independent *t* test was conducted to compare the mean changes in parameters between the groups after 12 week supplementation with WPC and SPI preloads. Power analysis was carried out to assess the sample size for non significant values. Statistical power lower than 0.8 indicates the nonsignificance of values was due to low sample size. Values are presented as means  $\pm$  SD and statistical significance was considered at  $P < 0.05$ .

### 3. Results

#### 3.1. Study population and compliance

Of 52 qualified individuals commenced the study, 7 in SPI group quitted the study within the first 2 weeks due to gastrointestinal discomfort. The results were reported on the 45 individuals who completed the study (WPC: 26 men; SPI: 19 men). Baseline characteristics of participants were not significantly different between the groups (Table 1). PA remained unchanged between groups when compared to baseline (Data are not shown).

Supplements were well tolerated with 94.9% and 93.9% compliances in terms of WPC and SPI consumption respectively.

After the intervention, data of 180 questionnaires of 24 h recall and PA, 315 questionnaires of BP and 90 questionnaires of FBS, TC, TG, LDL, HDL, VLDL, apo AI and apo B were analyzed.

**Table 1**

General characteristic of the participants in WPC and SPI groups.<sup>c</sup>

	WPC <sup>a</sup>	SPI <sup>b</sup>	P-value <sup>*</sup>
Number	26	19	0.8
Age ( $\bar{x} \pm$ SD)	39.4 $\pm$ 6.9	38.8 $\pm$ 8.8	0.8
Height (cm)	171.1 $\pm$ 7.4	171.8 $\pm$ 8.5	0.77
Body weight (Kg)	93.9 $\pm$ 11.5	95.2 $\pm$ 12.9	0.72
BMI (Kg/m <sup>2</sup> )	32.1 $\pm$ 3.2	32.1 $\pm$ 2.7	0.9

<sup>\*</sup>P-values refer to comparisons between groups (independent *t*-test).

<sup>a</sup> Whey protein concentrate (WPC) (n = 26).

<sup>b</sup> Soy protein isolate (SPI) (n = 19).

<sup>c</sup> Values are mean  $\pm$  SD.

#### 3.2. Dietary intake

Nutrient intakes were not significantly different between the groups prior to the intervention (Table 2). Calorie, protein and carbohydrate intake decreased significantly in both groups (respectively  $p = 0.045$ ,  $p = 0.21$ ,  $p = 0.06$ ) as a consequence of appetite and CI reduction (Data are not shown). Prior to the study, dietary protein intake of participants in WPC group was 14.51% of CI which increased to 16.12% of CI because of CI reduction. With respect to the protein of WPC (17.37% of CI), total protein intake increased to 33.5% of CI at the study completion. As well, in SPI group, dietary protein intake was 15.46% at the beginning and decreased to 15.03% of CI at the end of the study. Regarding the protein of SPI (13.65% of CI), total protein intake increased to 28.7% of CI. At week 12, WPC and SPI preloads lowered CI significantly ( $p < 0.001$  and  $p < 0.028$  respectively).

#### 3.3. Blood pressure

After intervention, individuals receiving WPC and SPI revealed significant mean changes of SBP and DBP values between the groups ( $p < 0.02$  and  $p = 0.001$  respectively) (Table 3). After 12 weeks, within group mean changes of SBP and DBP (–16.6 mmHg, –7 mmHg respectively) in WPC group and within mean changes of SBP (1.5 mmHg) and of DBP (–1.9 mmHg) in the SPI group were nonsignificant and significant respectively (Table 3).

#### 3.4. Fasting blood sugar

After 12 weeks, mean changes of FBS was not significant between the groups ( $p = 0.44$ ) (Table 3). Within group mean changes of FBS in WPC group (–9.2 mg/dL) and in SPI group (–5 mg/dL) were significant ( $p < 0.001$ ) and nonsignificant ( $p = 0.39$ ) respectively when compared to baseline (Table 3). In both groups, there was impaired fasting glucose at baseline which was improved at the end of the intervention. Although not statically significant between the groups, mean changes of FBS were clinically significant at week 12. The test power value observed was equal to 0.118 which was lower than the regarded value and the nonsignificant difference between the groups for FBS might be due to the low test power.

#### 3.5. Lipid profile

Data of lipid profile evaluation results of participants are presented in Table 3. After 12 weeks, the mean changes of apo AI and apo B were significant between the groups ( $p < 0.001$ ). The within group mean changes from baseline in apo AI of WPC group (17.1 mg/dL) was significant ( $p < 0.001$ ) but in SPI group this change (0.9 mg/dL) was not significant ( $p = 0.81$ ). After 12 weeks, the within group mean changes from baseline in apo B of WPC group

**Table 2**  
Mean and SD of nutrient intakes of participants before and after the study<sup>c</sup>.

	Groups	Before ( $\bar{X} \pm SD$ )	After ( $\bar{X} \pm SD$ )	*P-value	<sup>†</sup> P-value
Calorie (Kcal)	WPC <sup>a</sup>	2462.5 $\pm$ 621.9	1243.06 $\pm$ 303.95	0.001	
	SPI <sup>b</sup>	2235.1 $\pm$ 912	1582.95 $\pm$ 613.58	0.028	0.045
Protein(gr)	WPC	89.35 $\pm$ 27.57	50.13 $\pm$ 21.76	<0.001	
	SPI	86.39 $\pm$ 19.73	59.56 $\pm$ 16.53	<0.001	0.21
Carbohydrate (gr)	WPC	372.78 $\pm$ 87.57	187.15 $\pm$ 54.38	<0.001	
	SPI	353.83 $\pm$ 115.75	243 $\pm$ 80.17	0.009	0.006
Fat (gr)	WPC	89.35 $\pm$ 27.57	50.13 $\pm$ 21.76	<0.001	
	SPI	70.5 $\pm$ 30.02	52.4 $\pm$ 20.88	0.045	0.09
Cholesterol (mg)	WPC	251.99 $\pm$ 152.23	195.56 $\pm$ 418.47	0.4	0.37
	SPI	222.13 $\pm$ 125.5	255.77 $\pm$ 148.47	0.4	
Saturated fatty acid (gr)	WPC	20.84 $\pm$ 152.23	10.04 $\pm$ 3.37	<0.001	
	SPI	20.44 $\pm$ 7.5	14.6 $\pm$ 7.1	0.02	0.09
Poly unsaturated fatty acid(gr)	WPC	21.83 $\pm$ 16.87	10.76 $\pm$ 6.1	0.002	0.23
	SPI	18.5 $\pm$ 10.18	12.9 $\pm$ 7.8	0.088	
Mono unsaturated fatty acid(gr)	WPC	23.11 $\pm$ 11.74	11.22 $\pm$ 4.5	<0.001	0.19
	SPI	19.79 $\pm$ 7.6	77.11 $\pm$ 148.76	0.36	
Vitamine A (RE)	WPC	583.35 $\pm$ 238.3	480.94 $\pm$ 229.4	0.35	0.67
	SPI	813.06 $\pm$ 840.67	836.42 $\pm$ 388.3	0.13	
Vitamine C (mg)	WPC	164.05 $\pm$ 128.22	63.42 $\pm$ 19.9	<0.001	0.24
	SPI	145.4 $\pm$ 128.03	93.4 $\pm$ 73.68	0.14	
Vitamine E (mg)	WPC	20.19 $\pm$ 23	9.9 $\pm$ 5.12	0.025	0.33
	SPI	16.94 $\pm$ 9.83	12.22 $\pm$ 8.25	0.15	
Calcium (mg)	WPC	960.19 $\pm$ 342.61	507.15 $\pm$ 168.53	<0.001	0.7
	SPI	1025.6 $\pm$ 325.94	617.485 $\pm$ 203.31	<0.001	
Fiber (gr)	WPC	12.3 $\pm$ 8.9	9.2 $\pm$ 4.8	0.1	0.7
	SPI	12.24 $\pm$ 8.9	10.34 $\pm$ 3.96	0.39	

\*P-values refer to comparisons between week 0 and week 12 within groups (Paired t-test).

<sup>†</sup>P-values refer to comparisons between groups (independent t-test).<sup>a</sup> Whey protein concentrate (WPC) (n = 26).<sup>b</sup> Soy protein isolate (SPI) (n = 19).<sup>c</sup> Values are mean  $\pm$  SD.

(−16.8 mg/dL) was significant but in SPI group (2.8 mg/dL) was not significant ( $p = 0.81$ ). Before the study, apo AI was significantly different between the groups which evaluated by ANCOVA showed that the results were not affected by this difference and therefore WPC supplementation on apo AI was more effective.

As it is shown in Table 3, at week 12, the mean changes of TC was not significant between the groups ( $p = 0.22$ ). Within group mean changes of TC in WPC group (−16.8 mg/dL) were significant ( $p = 0.001$ ) but these mean changes in SPI group (−7.6 mg/dL) were nonsignificant ( $p = 0.22$ ) when compared to baseline. In both groups, TC was higher than the borderline at baseline which decreased in WPC. Although statistically nonsignificant, this decrease was clinically significant in WPC compared to SPI. The test power value observed was equal to 0.227 which was lower than regarded value and the nonsignificant difference between the groups for TC might be due to low test power.

At week 12, nonsignificant mean changes of VLDL and TG ( $p = 0.19$ ) and significant mean changes of LDL, HDL ( $p < 0.015$  and  $p < 0.017$  respectively) were observed between the groups. Within group mean changes from baseline in VLDL and TG of WPC group (−8.2 mg/dL and −41.2 mg/dL respectively) were significant ( $p < 0.001$ ) but in SPI group within mean changes (−3.6 mg/dL and −18.6 mg/dL respectively) were not significant ( $p = 0.27$ ). The test power value observed for VLDL and TG was equal to 0.255 which was lower than regarded value and the nonsignificant difference between the groups for VLDL and TG might be due to low test power. Before the study TG in WPC group was high and in SPI was in high borderline which decreased after 12 weeks. Although this decrease was nonsignificant statistically between the groups, the within group mean changes in WPC group were clinically significant compared to SPI group.

After 12 weeks, the mean changes from baseline in LDL and HDL of WPC group (−13.5 mg/dL and 4.3 mg/dL respectively) were significant ( $p < 0.001$  and  $p = 0.001$  respectively) but in SPI group

mean changes (−0.9 mg/dL and 0.38 mg/dL respectively) were not significant ( $p = 0.86$  and  $p = 0.73$  respectively). Before the study, HDL was significantly different between the groups which evaluating with ANCOVA showed that the results were not affected by this difference and therefore WPC supplementation on HDL was more effective.

#### 4. Discussion

The results of this 12-week randomized, double blind clinical trial in free living overweight and obese men indicate that WPC exerted stronger effects on decreasing SBP, DBP, FBS and on improving lipid profile of participants than SPI did. Accordingly, we confirmed the research hypothesis that supplemental preloads of WPC and SPI at 30 min before the largest meal of overweight and obese men would decrease SBP, DBP, FBS and improve lipid profile of participants although WPC was stronger than SPI.

BP in the present study changed in consistent with a previous study in overweight and obese individuals [25]. In contrast, in a study in NASH patients, whey consumption led to nonsignificant increase of SBP and DBP [20] which is likely due to low dose of supplement and high degree of oxidative stress in the patients unlike our health participants. In other clinical trials, BP revealed no change after receiving 35 gr soy nuts by women with metabolic syndrome [23] or after 30 gr ingesting soy protein containing 132 mg phytoestrogen by postmenopausal women with type 2 diabetes [31]. These disagreements may result from low dose of soy protein and low BP of participants. Overall, in the present study the amount of isoflavones were not enough to influence the BP and BP reduction observed here seem to be a result of the protein intake itself [32].

Mean FBS decreased in WPC significantly and in SPI nonsignificantly. Interestingly, this finding was similar to a previous study [33] but unlike our participants with ad libitum food intake, in that



**Table 3**Effects of 2-wk supplementation with WPC and SPI preloads on metabolic indices before and after the study<sup>m</sup>.

	Groups	Before ( $\bar{X} \pm \text{SD}$ )	After ( $\bar{X} \pm \text{SD}$ )	Mean changes	*P-value
SBP <sup>c</sup> (mmHg)	WP <sup>a</sup>	131.3 $\pm$ 11.6	114.7 $\pm$ 22.4	–16.6 $\pm$ 10.8	0.001
	SPI <sup>b</sup>	118.8 $\pm$ 29.3	120.3 $\pm$ 11.4	1.5 $\pm$ 17.9	0.81
<i>p</i> -value <sup>†</sup>				<0.02	
DBP <sup>d</sup> (mmHg)	WPC	83.1 $\pm$ 16	76.1 $\pm$ 14.2	–7 $\pm$ 1.8	<0.001
	SPI	80.8 $\pm$ 8	78.9 $\pm$ 6.6	–1.9 $\pm$ 1.4	0.031
<i>p</i> -value <sup>†</sup>				0.001	
FBS <sup>e</sup> (mg/dl)	WPC	103.1 $\pm$ 10.8	94.1 $\pm$ 9.5	–9 $\pm$ 1.3	<0.001
	SPI	107.4 $\pm$ 48.1	102.4 $\pm$ 42.4	–5 $\pm$ 5.7	0.39
<i>p</i> -value <sup>†</sup>				0.44	
Apo A-I <sup>f</sup> (mg/dl)	WPC	135.3 $\pm$ 12.1	152.4 $\pm$ 12.1	17.1	<0.001
	SPI	144.9 $\pm$ 19.2	145 $\pm$ 3.7	0.9 $\pm$ 15.5	0.81
<i>p</i> -value <sup>†</sup>				<0.001	
Apo B <sup>g</sup>	WPC	135.3 $\pm$ 12.1	152.4 $\pm$ 12.1	17.1	<0.001
	SPI	144.9 $\pm$ 19.2	145.8 $\pm$ 3.7	0.9 $\pm$ 15.5	0.81
<i>p</i> -value <sup>†</sup>				<0.001	
TC <sup>h</sup> (mg/dl)	WPC	200.5 $\pm$ 32.2	183.7 $\pm$ 30.9	–16.8 $\pm$ 1.3	0.001
	SPI	210.2 $\pm$ 36.8	202.6 $\pm$ 38.8	–7.6 $\pm$ 2	0.22
<i>p</i> -value <sup>†</sup>				0.22	
VLDL <sup>i</sup> (mg/dl)	WPC	40.9 $\pm$ 12.2	32.7 $\pm$ 11.1	–8.2 $\pm$ 1.1	<0.001
	SPI	38.9 $\pm$ 18.4	35.3 $\pm$ 13.5	–3.6 $\pm$ 4.9	0.27
<i>p</i> -value <sup>†</sup>				0.19	
LDL <sup>j</sup> (mg/dl)	WPC	123.4 $\pm$ 24.7	109.9 $\pm$ 22.8	–13.5 $\pm$ 1.9	<0.001
	SPI	127.6 $\pm$ 29.2	128.5 $\pm$ 32.9	0.9 $\pm$ 3.7	0.86
<i>p</i> -value <sup>†</sup>				0.015	
HDL <sup>k</sup> (mg/dl)	WPC	38.2 $\pm$ 3.9	42.5 $\pm$ 7.6	4.3 $\pm$ 3.7	0.001
	SPI	41.82 $\pm$ 6.6	42.2 $\pm$ 6.8	0.38 $\pm$ 0.6	0.73
<i>p</i> -value <sup>†</sup>				0.017	
TG <sup>l</sup> (mg/dl)	WPC	204.8 $\pm$ 60.9	163.6 $\pm$ 55.5	–41.2 $\pm$ 5.4	<0.001
	SPI	194.9 $\pm$ 91.9	176.3 $\pm$ 67.4	–18.6 $\pm$ 24.5	0.27
<i>p</i> -value <sup>†</sup>				0.19	

Groups Before ( $\pm$ SD) After ( $\pm$ SD) Mean changes \*P-value.<sup>\*</sup>P-values refer to comparisons between week 0 and week 12 within groups (Paired t-test).<sup>†</sup>P-values refer to comparisons between groups (independent t-test).<sup>a</sup> Whey protein concentrate (WPC) (n = 26).<sup>b</sup> Soy protein isolate (SPI) (n = 19).<sup>c</sup> Systolic blood pressure (SBP).<sup>d</sup> Diastolic blood pressure (DBP).<sup>e</sup> Fasting blood sugar (FBS).<sup>f</sup> Apolipoprotein AI (Apo AI).<sup>g</sup> Apolipoprotein B (Apo B).<sup>h</sup> Total cholesterol (TC).<sup>i</sup> Very low density lipoprotein (VLDL).<sup>j</sup> Low density lipoprotein (LDL).<sup>k</sup> High density lipoprotein (HDL).<sup>l</sup> Triglyceride (TG).<sup>m</sup> Values are mean  $\pm$  SD.

study calorie intake was reduced by 1050 KJ to prevent possible weight gain which might influence the results. In addition, comparing whey with glucose is definitely effective on the significant results. The main reason of blood glucose decrease by whey protein, is its low glycemic index and carbohydrate content, amino acids stimulating insulin or incretin hormone secretion and gastric emptying [34]. In another study FBS did not change significantly [35], which was likely due to the short duration of the study (4 weeks compared with this 12 week intervention). From 1910 that the beneficial effects of soy protein on diabetes proposed for the first time [36], limited studies on diabetic patients show that this effect is due to soluble fiber contents in soy bean [37]. According to a previous study soy protein ingestion led to significant reduction in insulin resistance and HbA1c and to a significant increase in FBS [31], in which similar to ours, soy protein lacked soluble fiber but the amount of isoflavone intake was higher than our study. Furthermore, studies demonstrate that many metabolic effects of soy protein are specific to women but whether a sex effect is evident for glycemic control is unclear [31]. A reason for no significant difference in FBS of SPI group might be due to higher insulin resistance in the morning resulting from fasting period during

the night [38] whilst BCAAs of whey can stimulate insulin more than soy. As well, WPC decreased carbohydrate intake more than SPI. Additionally, high levels of cysteine in whey protein increases taurin synthesis in liver [39] which can increase mRNA of apo A-I [40]. After the intervention, the increase in average apo A-I was significant in WPC group and was nonsignificant in SPI group and the mean changes between the groups were significant. To our knowledge, no human study has investigated the effect of whey protein on apo A-I on obese individuals but regarding the inverse association between apo A-I, the main protein of HDL, and obesity [41], the increase in apo A-I by whey protein can be due to much decrease of abdominal obesity in this group (data not shown) and also due to sulfuric amino acids of whey protein. Similarly, in a previous randomized double blind crossover [42], lack of significant changes in apo A-I may result from lower levels of apo A-I at baseline in soy group when comparing with the present study. In contrast, apo A-I increase in another study [23] may be a result of higher intakes of fiber, isoflavone and unsaturated fatty acids by soy protein ingestion.

At the end of the study, the mean changes of apo B in whey group decreased significantly and increased nonsignificantly in SPI

group and mean changes between the groups were significant but Pipe et al. observed no significant effects on apoB despite of significant decrease of apoB/apo A-I ratio [42] whereas in another study, supplementation with soy protein decreased apo B100 significantly [23] that can be attributable to higher apo B100 at baseline (150 mg/dL) compared with lower level (105.3 mg/dL) in the current study which lead to better results after soy supplementation.

TC decreased significantly in WPC but nonsignificantly in SPI group. In accordance with this finding, some other studies reported a significant reduction in TC after supplementation with whey [25,35]. However, in a study TC did not change [21], which may be due to lower levels of cholesterol at baseline, short term of the intervention, low dose of protein and not consuming the supplement before the meal to provide plenty time for BCAA of whey protein to exert favorable effects. In contrast, evidence show, 30 gr consumption of SPI [31,43] was able to lower TC significantly. High amounts of leucin in whey protein may contribute to TC reduction which data on molecular mechanism is limited [44]. Soy protein through its high concentration of arginin [45] and also through promoting excretion of cholesterol and bile acids in feces, can decrease blood cholesterol [46].

After completing the study, VLDL in WPC group decreased significantly and in SPI group nonsignificantly. The reason behind the inconsistency of these results with previous one [23] may result from some sex dependant effects of soy protein [31]. LDL in WPC group at study completion decreased significantly and in SPI group increased nonsignificantly. Mean changes of LDL between two groups were significant. This finding was in agreement with another research [25]. But nonsignificant reduction of LDL [20] or no change of it [21] in some studies may be attributed to low dose of protein intake and not consuming the supplement before meals. Unlike the current study, soy protein used in other studies led to significant decrease in LDL [23,31,42] which may result from low contents of fiber, isoflavone and unsaturated fatty acids in SPI in this study.

After this intervention, the observed increase of HDL was significant in WPC group and nonsignificant in SPI group. The mean changes between the groups was significant. Lack of significant changes of HDL level in other researches [25,35] may be induced by higher HDL levels compared to low HDL levels in the present study and in a similar one [42] and also short term supplementation and low dose of whey intake before the meal. Similarly, in some other studies soy intake caused a significant increase in HDL [43]. This, can be explained by sulfuric amino acids like metionin, cystein and taurin that are able to increase apo A-I and decrease apo B which increase HDL and decrease LDL and VLDL respectively [47].

High concentration of plasma TG is often associated with low HDL concentration [48]. At the end of the study, TG decreased significantly in WPC group and nonsignificantly in SPI group. The same results in other studies [25,36] are reported that can be explained by decrease of glucagon to insulin ratio and by decrease in carbohydrate and fat intake after whey consumption [36]. Confirming this finding, we can point to the studies in which TG decreased by 30 gr soy protein consumption [43,49] that is inconsistency with other studies [31,38]. The mechanism of the effect of whey protein on TG metabolism is still unknown. TG metabolism is influenced by factors including: intestinal secretion of chylomicrons, hepatic secretion of VLDL and uptake of lipoproteins by tissues lacking TG [50] and probably whey protein affecting one of these stages, lowers TG. Based on a meta-analysis by Zhan et al. of data from 23 studies, soy protein with isoflavone reduces TC, LDL and TG significantly and the magnitude of these effects is not only dose dependent but also is associated with the length of supplementation period and baseline concentration of cholesterol [51]. Another possible reason of not improving serum lipids in soy

group can be attributed to the individual differences in intestinal absorption of isoflavones. Isoflavone equol is not produced at the same amount in all people in response to soy protein intake and this is of importance in various effects of soy protein on serum lipids [52] which seems that this effect of whey is independent of weight and body fat decrease [53].

Major strengths of present study include: it was a randomized double blinded design and conducting it in participants' workplace which caused a precise monitoring the intervention. Given that different proteins exert different effects depending on dose, source and intake time, we determined the largest meal which was lunch, and supplemented participants with a complete dose of 65 gr WPC and 60 gr SPI equal to 52 gr effective material as protein, 30 min before lunch for 12 weeks. Unlike previous studies that evaluated the effects of substituting dietary proteins from mixed protein sources [54–57], this study revealed beneficial effects of WPC and SPI preloads on Bp, FBS and lipid profile of participants. Besides, comparing two different sources of proteins in this study helpt us rule out the effect of high protein diets on metabolic factors as a confounding factor. Because we were interested to evaluate biological impacts of soy protein, not its non protein components, and since isoflavone contents of isolated forms of soy are much lower, we so used SPI. Hence, these results were due to isoflavone free components of SPI including its proteins. On the other hand, according to literature review, there are limited studies on the impact of long time whey and soy protein preloads on apo A-I in free living overweight and obese individuals. Limitations of this study that are suggested to be taken into consideration in future studies are not measuring plasma amino acids before and after the intervention.

In conclusion, the hypothesis was confirmed and supplemental preloads of WPC and SPI have been shown to decrease BP and FBS and to modify lipid profile of healthy overweight and obese men in their free living conditions, although WPC exerted stronger impacts.

## 5. Conclusion

This study demonstrated that long term supplemental preloads with WPI and SPI can improve metabolic risk factors associated in overweight and obese individuals though the effects of WPC were higher. The first major finding was that beneficial effects of WPC and SPI liquid preloads on metabolic risk factors in overweight and obese individuals are independent of body weight and body composition changes as food intake and body weight decreased in both groups (data not presented). Another major finding is that favorable changes of metabolic risk factors by SPI not only depend on its isoflavones and fiber but also on beneficial amino acids of soy protein.

## Competing interest

The authors declare that they have no competing interests.

## Authors' contributions

AT and MV: conceived and designed the study; AT: conducted the trial and collected the data, wrote the manuscript and analyzed the data, MV: supervised the conduct of the trial and data collection, provided advice and edited the manuscript; MG: analyzed and interpreted the data; FS: supervised the conduct of the study, provided advice and edited the manuscript; IH: assisted with study design, clinical assessment and conduct of the study. All authors contributed intellectually to the manuscript and all reviewed drafts and accepted the final draft. All authors read and approved the final manuscript.

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